

# Amino acid availability from animal, blended, and plant feedstuffs for hybrid striped bass (*Morone chrysops* × *M. saxatilis*)

T.G. GAYLORD<sup>1</sup>, S.D. RAWLES<sup>1</sup> & D.M. GATLIN III<sup>2</sup>

<sup>1</sup> United States Department of Agriculture/Agriculture Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR, USA; <sup>2</sup> Department of Wildlife and Fisheries Sciences and Faculty of Nutrition, Texas A & M University System, College Station, TX, USA

## Abstract

The refinement of hybrid striped bass feed formulations has been progressing rapidly. There is still, however, limited information available regarding nutrient digestibility and amino acid availability from common feedstuffs. A pair of experiments was conducted to determine the protein digestibility and amino acid availability to sunshine bass from an assortment of commercially available animal protein feedstuffs, blended animal products and plant protein feedstuffs in extruded diets. The feedstuffs tested were blood meal, poultry by-product meal, fish solubles, Pro-Pak 60, Pro-Pak 65, ProCon 65RDB, and 60FMC for the animal protein feedstuffs and brewer's yeast, canola meal, peanut meal, and sunflower meal for the plant protein feedstuffs. Test diets consisted of a mixture of nutritionally complete reference diet and test ingredient. Triplicate tanks of fish were fed their respective diets for 7 days prior to collection of faeces by stripping. Apparent digestibility coefficients of protein (ADC-CP) in the animal products ranged from 47% for 60FMC to a high of 70% for fish solubles. ADC-CPs for animal products were not different across products. Blood meal, poultry by-product meal, Pro-Pak 60, Pro-Pak 65, and ProCon had intermediate ADC-CPs of 63, 55, 63, 57 and 52%, respectively. ADC-CP in plant feedstuffs ranged from 43% for canola meal to 80% for peanut meal. Brewer's yeast, canola meal, and sunflower meal had intermediate ADC-CPs at 54, 43 and 69%, respectively. Apparent amino acid availability coefficients were variable across animal products and did not necessarily correlate to the ADC-CPs for a given feedstuff. Isoleucine availability was low in blood meal at 38% compared with 59% or better for the remaining amino acids. Lysine, tyrosine and phenylalanine availability from fish solubles was low at 31, 35 and 44%, respectively. Amino

acid availability from Pro-Pak 60 was consistently higher across all amino acids for the animal products and blends tested. Of the plant products tested, peanut meal was the best performing feedstuff relative to amino acid availability.

**KEY WORDS:** amino acid availability, digestibility, hybrid striped bass

Received 19 September 2003, accepted 14 May 2004

Correspondence: Steven D. Rawles, United States Department of Agriculture/Agriculture Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, PO Box 1050, 2955 Highway 130 East, Stuttgart, AR 72160-1050, USA. E-mail: srawles@spa.ars.usda.gov

## Introduction

A wealth of information on the nutritional needs of hybrid striped bass is accumulating as the industry expands (Gatlin 1997). This has allowed the formulation of more accurate diets to meet the nutritional needs of hybrid striped bass in various production scenarios. Quality fishmeals are still the preferred source of protein, thus amino acids, in diets for hybrid striped bass. As the price of fishmeal continues to rise, it will become increasingly lucrative to develop alternate sustainable protein sources for fish diets. Although any number of factors may influence the availability of amino acids from different batches of the same ingredient, data on amino acid availability is necessary to accurately formulate diets. The amino acid requirements for lysine, methionine, threonine and arginine have been quantified for hybrid striped bass (Griffin *et al.* 1992, 1994; Keembiyehetty & Gatlin 1992, 1993, 1997) while requirements for the remaining six essential amino acids have been estimated based on an ideal amino acid ratio (Brown 1995) or the requirements of

similar species [National Research Council (NRC) 1993]. The following experiments were undertaken to determine the apparent amino acid availability coefficients for a group of feedstuffs readily available to feed companies.

## Materials and methods

### Diets

The indirect method was used with chromic oxide as the indigestible marker to determine the protein digestibility and amino acid availability from eleven readily available feedstuffs (Table 1) for hybrid striped bass. The test ingredients included products from animal sources, products that were commercially blended and often used as fishmeal replacements in aquaculture feeds, and products that were from plant sources. The animal products consisted of ring dried blood meal, poultry by-product meal, and fish solubles. The commercially blended products consisted of Pro-Pak<sup>TM</sup>, Pro-Pak-65<sup>TM</sup>, ProCon<sup>TM</sup> 65RDB and 60FMC<sup>TM</sup>. Exact composition of the commercial blends is proprietary; however, information obtained from the respective suppliers indicates they are blends of animal and marine by-products without vegetable proteins that may or may not include supplemental amino acids. The plant products tested consisted of brewer's yeast, canola meal, peanut meal, and solvent extracted, dehulled sunflower meal 35.

The reference diet (Table 2) was formulated to resemble a commercial diet which met or exceeded all known nutritional requirements of hybrid striped bass (Gatlin 1997). Test diets consisted of a 70 : 30 mixture of reference diet to test ingredient. All dry ingredients were ground to less than 0.5 mm in a hammermill (Model LM6; Kelly Duplex Mill and Manufacturing, Inc., Springfield, OH, USA). Approximately 5% of the total dry ingredients in each diet, including vitamin and mineral packs, were V-mixed (P-K Blend Master<sup>1</sup>; Patterson-Kelley, Inc., East Stroudsburg, PA, USA) for 15 min then blended with the remaining ingredients for 30 min in an electric cement mixer (Sears, Roebuck & Co., Hoffman Estates, IL, USA) prior to extrusion. Diets were extruded on a twin screw extruder (Wenger X-20; Wenger Manufacturing, Inc., Sabetha, KS, USA) at the Texas A&M Food Protein Research and Development Center. The conditions of extrusion are as follows: diet

material was introduced manually to the pre-conditioner and heated to 77 °C with the addition of water and steam at 0.147 kg min<sup>-1</sup>. Conditioner cylinder speed was set at 150 rpm. Material was introduced into the extruder at a rate (feeder) of 20 rpm. Extruder barrel temperatures were 38–39 °C (zone 2), 79–80 °C (zone 3), 80 °C (zone 4) and 65–66 °C (zone 5). Extruder main driveshaft speed varied from 335–360 rpm at 50–58 amps. Material exited the extruder through at 5 mm die and was subsequently dried to less than 12% moisture on a horizontal bed dryer (Wenger 360 Dryer/Cooler; Wenger Manufacturing, Inc.) at 68–99 °C. Diets were then transported, stored, and fed at the USDA/ARS Harry K. Dupree Stuttgart National Aquaculture Research Center.<sup>2</sup>

### Fish and sample collection

Reciprocal cross hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂) were obtained from a commercial producer (Keo Fish Farms, Keo, AR, USA) and stocked at a rate of 100, 75 g fish per 600-L fibreglass tank. Water temperature was maintained at 23 °C throughout the experiment using flow-through well water. Lighting was maintained on a 12 : 12 h diurnal cycle. Three tanks of fish were randomly assigned to each diet. Test diets were divided into two trials over time with the animal and blended product diets being fed and faeces collected first, followed by a 1-week recovery period. In the subsequent experiment, the plant product diets were fed according to the same protocol. The reference diet was fed during both trials. Diets were fed once daily to apparent satiation for 6 days prior to the first faecal collection and faecal matter was collected by manual stripping approximately 7 h post-feeding on the seventh day. Manual stripping of fish was accomplished by gently netting all fish in one pass of the tank, sequentially removing fish without anaesthesia, and gently drying then applying pressure to the lower abdominal region to express faecal matter into a plastic weighing pan. Care was taken to exclude urinary excretions from the collection. After stripping, fish were given a saline (15–20 ppt) bath for 10–15 min to reduce handling stress and the potential for secondary infections then returned to their culture tank. A second faecal collection was performed after an additional 2 days of feeding. Faecal samples for a given tank were dried overnight at 50 °C, pooled for the 2 days of

<sup>1</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

<sup>2</sup> All programmes and services of the US Department of Agriculture are offered on a non-discriminatory basis without regard to race, colour, national origin, religion, sex, age, marital status or handicap.

**Table 1** Analysed protein and amino acid composition of the feedstuffs tested

	International feed number	Protein g kg <sup>-1</sup> dry	g kg <sup>-1</sup> of crude protein									
			ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL
Animal Products and Blends												
Blood meal, ring dried <sup>1</sup>	5-00-381	968.1	40.9	129.8	5.7	146.7	101.0	13.6	77.3	47.2	29.3	103.8
Poultry by-product meal <sup>2</sup>	5-03-798	667.0	68.4	37.3	41.2	68.6	45.6	18.6	39.6	41.0	30.9	59.2
Pro-Pak™ 1		668.0	75.1	44.2	38.6	79.2	51.3	26.3	46.1	44.5	29.5	73.9
Pro-Pak-65™ 1		749.0	67.7	64.6	35.2	99.2	66.5	43.4	59.9	52.8	33.2	91.9
ProCon™ 65RDB 3		713.0	53.9	67.5	20.0	106.3	69.3	15.0	55.6	40.9	26.6	75.7
60FMC™ 3		642.0	75.0	54.2	36.3	83.9	54.0	17.2	48.0	46.0	30.8	73.2
Fish solubles <sup>4</sup>		674.0	51.1	25.1	25.5	49.5	36.1	19.5	34.5	30.7	10.7	40.0
Plant products												
Brewer's yeast <sup>5</sup>	7-05-527	157.0	59.0	50.7	38.1	81.0	38.9	8.4	44.6	47.7	30.4	59.4
Canola meal <sup>6</sup>	5-06-145	424.0	69.4	54.6	49.8	75.8	61.9	14.6	45.1	48.0	31.9	65.5
Peanut meal <sup>7</sup>	5-03-650	509.0	117.2	45.2	41.0	68.7	37.4	7.4	56.7	32.1	39.7	51.5
Sunflower meal 35, solvent extracted, dehulled 6	5-04-739	392.0	82.8	44.1	48.6	65.0	38.2	14.8	49.9	46.2	26.7	60.8

<sup>1</sup> H.J. Baker Brothers Inc., Stamford, CT, USA.<sup>2</sup> Tyson Foods Inc., Rogers, AK, USA.<sup>3</sup> Mid-South Milling Inc., Memphis, TN, USA.<sup>4</sup> Omega Protein Inc, Hammond, LA, USA.<sup>5</sup> Conway Grain Inc., Conway, AR, USA.<sup>6</sup> Archer Daniels Midland Company, Decatur, IL, USA.<sup>7</sup> Sessions Inc., Enterprise, AL, USA.

collection, and stored at -20 °C until chemical analyses were performed.

### Chemical analysis

Dry matter and ash analysis of ingredients, diets and faeces were performed according to standard methods (AOAC 1995). Chromium was determined in diets and faeces by inductively coupled plasma atomic absorption spectrophotometry (The Perkin-Elmer Corporation, Norwalk, CT, USA) following wet ashing with nitric acid (AOAC 1995). Protein (N × 6.25) was determined in ingredients, diets and faeces by the Dumas method (AOAC 1995) on a Leco nitrogen analyzer (FP428; LECO Corporation, St Joseph, MI, USA). Amino acids were analysed in ingredients, diets and faeces by high-performance liquid chromatography (HP1100; Agilent Technologies, Wilmington, DE, USA) following acid hydrolysis (AOAC 1995) using pre-column *o*-phthaldehyde derivatization (Fleming *et al.* 1992). Lipid was determined in the reference diet by chloroform-methanol extraction (Folch *et al.* 1957). Total energy was determined in the reference diet by adiabatic bomb calorimetry (Parr1281; Parr Instrument Company Inc., Moline, IL, USA).

Apparent digestibility coefficients of each nutrient in the test diet and ingredients were calculated according to the following equations (Kleiber 1961):

$$\text{ADCN}_{\text{diet}} = 100 - 100 \left\{ \frac{\% \text{ Cr in diet}}{\% \text{ Cr in faeces}} \times \frac{\% \text{ nutrient in faeces}}{\% \text{ nutrient in diet}} \right\}$$

$$\text{ADCN}_{\text{ingredient}} = \{(a + b)\text{ADCN}_t - (a)\text{ADCN}_r\}b^{-1}$$

where  $\text{ADCN}_i$  is the apparent digestibility coefficient of the nutrient in the test ingredient;  $\text{ADCN}_t$  the apparent digestibility coefficients of the nutrient in the test diets;  $\text{ADCN}_r$  the apparent digestibility coefficients of the nutrient in the reference diet;  $a = (1 - p) \times \text{nutrient content of the reference diet}$ ;  $b = p \times \text{nutrient content of the test ingredient}$ ;  $p$  is the proportion of test ingredient in the test diet.

### Statistical analyses

The assumption of homogeneity of variance of the response data ( $\text{ADCN}_i$ ) was first verified according to Fry (1993) by observing no discernible pattern in plots of standardized residuals with respect to fitted responses. The program PROC MIXED, SAS Software Version 7.00 (SAS Institute, Inc., Cary, NC, USA) was then used to conduct a one-way analysis of variance for a mixed effects model (Ott 1977) in which diet was defined as a fixed effect and tank within treatments was defined as a random effect. The response data for animal products was analysed separately from the plant products due to separate collection times of faecal matter. Analysis

**Table 2** Composition of reference diet

Ingredient	g kg <sup>-1</sup> (as-fed basis)
Menhaden fish meal, Select <sup>TM1</sup>	292.0
Soybean meal, 48% solvent extracted <sup>2</sup>	182.2
Cottonseed meal, 41% <sup>3</sup>	162.1
Rice bran <sup>4</sup>	129.1
Wheat <sup>5</sup>	106.2
Vitamin premix <sup>6</sup>	1.2
Mineral premix <sup>7</sup>	1.0
Choline chloride <sup>8</sup>	0.8
Chromium (III) oxide <sup>8</sup>	10.0
Celufil <sup>9</sup>	75.4
Menhaden fish oil <sup>10</sup>	40.0
Analysed composition	g kg <sup>-1</sup> (dry weight basis)
Crude protein	405.3
Lipid	113.3
Phosphorus	18.6
Energy	20.22 MJ kg <sup>-1</sup>

<sup>1</sup> Omega Protein Corp., Hammond, LA, USA.<sup>2</sup> Riceland Foods Inc., Stuttgart, AR, USA.<sup>3</sup> Mountaire Feeds Inc., North Little Rock, AR, USA.<sup>4</sup> Producer's Rice Inc., Stuttgart, AR, USA.<sup>5</sup> Conway Grain Inc., AR, USA.<sup>6</sup> Vitamin premix (g kg<sup>-1</sup> premix): ascorbyl-2-polyphosphate (Stay-C 35) (250), thiamin mononitrate (12.5), riboflavin (75), D-calcium pantothenate (62.5), pyridoxine HCl (25), cyanocobalamin (0.05), vitamin A palmitate (10), DL-alpha tocopherol acetate (200), cholecalciferol (0.05), menadione sodium bisulphite (80.65), biotin (1.25), folic acid (4.5), myo-inositol (125), nicotinic acid (125), celufil (28.51).<sup>7</sup> Trace mineral premix (g kg<sup>-1</sup> premix): cobalt carbonate (0.30), copper oxide (6.26), ferrous sulphate heptahydrate (149.33), manganous oxide (34.70), potassium iodide (2.62), sodium selenate (0.60), zinc oxide (248.97), celufil (557.23).<sup>8</sup> Sigma-Aldrich Company, St Louis, MO, USA.<sup>9</sup> USB Corporation, Cleveland, OH, USA.<sup>10</sup> Omega Protein Corp., Reedville, VA, USA.

consisted of testing for differences in digestibility/availability of a particular nutrient among ingredients and for differences among amino acid availabilities within a particular ingredient. Differences among treatment means ( $N =$  three tanks/treatment) of digestibility coefficients for protein, individual amino acid availability, and mean amino acid availability within and among test ingredients were determined using the Tukey–Kramer procedure for pair-wise comparisons (Tukey 1953; Kramer 1956). Treatment effects in all statistical analyses were considered significant at  $P < 0.05$ .

## Results

The diets were well accepted by the fish during this experiment. The effects of ingredients on diet consumption were not addressed in this study; hence, consumption data was not collected. Diets were fed in excess, however, to ensure fish

were consuming as much feed as they would over an hour period.

Apparent digestibility coefficients of protein (ADC-CP) in the animal and blended products ranged from 47% for 60FMC to a high of 70% for fish solubles (Table 3) and were not statistically different among products. Blood meal, poultry by-product meal, Pro-Pak 60, Pro-Pak 65, and Pro-Con had intermediate ADC-CPs of 63, 55, 63, 57 and 52%, respectively. ADC-CP in plant feedstuffs ranged from 43% for canola meal to 80% for peanut meal. Brewer's yeast and sunflower meal had intermediate ADC-CPs at 54 and 69%, respectively.

Apparent amino acid availability coefficients were variable among animal products and did not necessarily compare with the ADC-CPs for a given feedstuff. Isoleucine availability was low in blood meal at 38% compared with 59% or better for the remaining amino acids (Table 3). Availability of lysine, tyrosine and phenylalanine in fish solubles was low at 31, 35 and 44%, respectively, compared with the availability of the remaining amino acids and ADC-CP. All amino acids were more available from Pro-Pak 60 than from the other animal products and blends tested.

Among the plant products tested, peanut meal was the best performing feedstuff relative to amino acid availability. Lysine and methionine availabilities were notably higher than most other amino acid availabilities in brewer's yeast, peanut meal and sunflower meal.

## Discussion

Blood meal protein was 63% digestible by hybrid striped bass in the present trial which is comparable with values reported for rainbow trout (69%) and channel catfish (74%) (NRC 1993) but lower than has been reported for Korean rockfish (86–87%) (Lee 2002) and previously for hybrid striped bass (86%) (Sullivan & Reigh 1995). Differences in the estimated ADCs of protein may be due to various factors including differences in drying temperatures and duration of storage. Similar trends in amino acid availabilities were observed in blood meal fed to Korean rockfish (Lee 2002). The availability of isoleucine in bloodmeal to Korean rockfish, for example, appeared severely depressed compared with other amino acids. Isoleucine availability also was low for blood meal in the current trial and potentially could be due to the relatively high leucine and valine contents competing for transport sites in the gut. The importance of branched chain amino acid balance in diets for other fish species have been reported by Robinson *et al.* (1984) and Yamamoto *et al.* (2004).

**Table 3** Apparent protein digestibility and amino acid availability coefficients of animal, blended and plant protein feedstuffs (mean  $\pm$  SE)

	International AA feed number	mean	Protein	ARG	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL	$P > F^1$
<b>Animal products and blends</b>														
Blood meal, ring dried	5-00-381	65 $\pm$ 1	63 $\pm$ 1 <sup>CD</sup>	*70 $\pm$ 1 <sup>AB</sup> <sub>XYZ</sub>	66 $\pm$ 1 <sup>BC</sup> <sub>X</sub>	*38 $\pm$ 2 <sup>E</sup> <sub>Y</sub>	*71 $\pm$ 1 <sup>A</sup> <sub>XY</sub>	*73 $\pm$ 3 <sup>WXY</sup>	64 $\pm$ 1 <sup>C</sup> <sub>X</sub>	69 $\pm$ 1 <sup>AB</sup> <sub>W</sub>	*59 $\pm$ 2 <sup>D</sup> <sub>X</sub>	69 $\pm$ 0.4 <sup>AB</sup> <sub>X</sub>	64 $\pm$ 1 <sup>C</sup> <sub>X</sub>	0.0001
Poultry by-product meal	5-03-798	61 $\pm$ 7	*55 $\pm$ 9	*74 $\pm$ 5 <sup>XY</sup>	59 $\pm$ 8 <sup>X</sup>	60 $\pm$ 7 <sup>X</sup>	*67 $\pm$ 6 <sup>Y</sup>	61 $\pm$ 8 <sup>XY</sup>	*67 $\pm$ 7 <sup>X</sup>	62 $\pm$ 7 <sup>XY</sup>	58 $\pm$ 8 <sup>X</sup>	*67 $\pm$ 6 <sup>X</sup>	61 $\pm$ 7 <sup>X</sup>	0.8118
Pro-Pak™		87 $\pm$ 4	*63 $\pm$ 8 <sup>B</sup>	87 $\pm$ 3 <sup>A</sup>	90 $\pm$ 3 <sup>A</sup>	90 $\pm$ 4 <sup>A</sup>	92 $\pm$ 3 <sup>A</sup>	*97 $\pm$ 4 <sup>A</sup>	*94 $\pm$ 3 <sup>A</sup>	88 $\pm$ 4 <sup>A</sup>	86 $\pm$ 4 <sup>A</sup>	86 $\pm$ 3 <sup>A</sup>	86 $\pm$ 4 <sup>A</sup>	0.0013
Pro-Pak-65™		66 $\pm$ 4	*57 $\pm$ 5 <sup>D</sup>	70 $\pm$ 3 <sup>ABC</sup> <sub>XYZ</sub>	67 $\pm$ 3 <sup>ABCD</sup> <sub>X</sub>	65 $\pm$ 4 <sup>BCD</sup> <sub>X</sub>	69 $\pm$ 3 <sup>ABC</sup> <sub>XY</sub>	*76 $\pm$ 1 <sup>A</sup>	*76 $\pm$ 2 <sup>AB</sup> <sub>X</sub>	65 $\pm$ 3 <sup>BCD</sup> <sub>XY</sub>	61 $\pm$ 4 <sup>CD</sup> <sub>X</sub>	68 $\pm$ 3 <sup>ABCD</sup> <sub>X</sub>	65 $\pm$ 4 <sup>CD</sup> <sub>X</sub>	0.0139
ProCon™ 65RDB		58 $\pm$ 3	52 $\pm$ 2	62 $\pm$ 3 <sup>Z</sup>	65 $\pm$ 1 <sup>X</sup>	*50 $\pm$ 5 <sup>XY</sup>	62 $\pm$ 3 <sup>Y</sup>	53 $\pm$ 6 <sup>XYZ</sup>	62 $\pm$ 3 <sup>X</sup>	56 $\pm$ 2 <sup>XYZ</sup>	58 $\pm$ 1 <sup>X</sup>	60 $\pm$ 3 <sup>X</sup>	58 $\pm$ 1 <sup>X</sup>	0.0645
60FMC™		57 $\pm$ 5	*47 $\pm$ 9	*64 $\pm$ 3 <sup>YZ</sup>	63 $\pm$ 3 <sup>X</sup>	54 $\pm$ 6 <sup>XY</sup>	60 $\pm$ 5 <sup>Y</sup>	51 $\pm$ 10 <sup>YZ</sup>	62 $\pm$ 7 <sup>X</sup>	52 $\pm$ 6 <sup>YZ</sup>	56 $\pm$ 4 <sup>X</sup>	58 $\pm$ 5 <sup>X</sup>	66 $\pm$ 3 <sup>X</sup>	0.4774
Fish solubles		66 $\pm$ 4	70 $\pm$ 7 <sup>ABC</sup>	80 $\pm$ 5 <sup>WX</sup>	60 $\pm$ 5 <sup>ABCD</sup> <sub>X</sub>	51 $\pm$ 6 <sup>A</sup>	80 $\pm$ 1 <sup>A</sup>	*31 $\pm$ 12 <sup>E</sup>	74 $\pm$ 7 <sup>AB</sup> <sub>X</sub>	*44 $\pm$ 3 <sup>DEF</sup> <sub>X</sub>	66 $\pm$ 4 <sup>ABCD</sup> <sub>X</sub>	*35 $\pm$ 11 <sup>F</sup> <sub>Y</sub>	53 $\pm$ 5 <sup>BCDE</sup> <sub>X</sub>	0.0002
	$P > F$		0.2548	0.0019	0.0015	0.0002	0.0003	0.0007	0.0051	0.0001	0.0036	0.0009	0.0023	
<b>Plant products</b>														
Brewer's yeast	7-05-527	73 $\pm$ 4	54 $\pm$ 5 <sup>CD</sup> <sub>X</sub>	75 $\pm$ 7 <sup>BC</sup> <sub>X</sub>	54 $\pm$ 7 <sup>CD</sup> <sub>XY</sub>	*49 $\pm$ 4 <sup>A</sup> <sub>W</sub>	69 $\pm$ 2 <sup>CD</sup> <sub>X</sub>	*110 $\pm$ 1 <sup>A</sup>	*94 $\pm$ 11 <sup>AB</sup> <sub>W</sub>	58 $\pm$ 13 <sup>CD</sup> <sub>X</sub>	67 $\pm$ 5 <sup>CD</sup> <sub>X</sub>	66 $\pm$ 2 <sup>CD</sup> <sub>X</sub>	*50 $\pm$ 5 <sup>D</sup> <sub>X</sub>	0.0001
Canola meal	5-06-145	48 $\pm$ 1	43 $\pm$ 1 <sup>C</sup> <sub>X</sub>	*61 $\pm$ 0.3 <sup>A</sup> <sub>X</sub>	42 $\pm$ 6 <sup>C</sup> <sub>Y</sub>	45 $\pm$ 1 <sup>A</sup> <sub>W</sub>	53 $\pm$ 1 <sup>AB</sup> <sub>Y</sub>	54 $\pm$ 6 <sup>AB</sup> <sub>X</sub>	*62 $\pm$ 1 <sup>A</sup> <sub>X</sub>	54 $\pm$ 3 <sup>AB</sup> <sub>X</sub>	54 $\pm$ 1 <sup>AB</sup> <sub>Y</sub>	54 $\pm$ 2 <sup>AB</sup> <sub>Y</sub>	*37 $\pm$ 1 <sup>C</sup> <sub>Y</sub>	0.0001
Peanut meal	5-03-650	85 $\pm$ 3	80 $\pm$ 4 <sup>C</sup> <sub>W</sub>	92 $\pm$ 2 <sup>AB</sup> <sub>W</sub>	*68 $\pm$ 4 <sup>D</sup> <sub>WX</sub>	83 $\pm$ 3 <sup>A</sup> <sub>W</sub>	88 $\pm$ 3 <sup>ABC</sup> <sub>W</sub>	94 $\pm$ 7 <sup>A</sup> <sub>W</sub>	94 $\pm$ 4 <sup>A</sup> <sub>W</sub>	88 $\pm$ 2 <sup>ABC</sup> <sub>W</sub>	81 $\pm$ 3 <sup>BC</sup> <sub>W</sub>	85 $\pm$ 2 <sup>ABC</sup> <sub>W</sub>	80 $\pm$ 3 <sup>C</sup> <sub>W</sub>	0.0019
Sunflower meal 35, solvent extracted-dehulled	5-04-739	79 $\pm$ 3	69 $\pm$ 6 <sup>C</sup> <sub>W</sub>	92 $\pm$ 2 <sup>W</sup>	72 $\pm$ 0.03 <sup>C</sup> <sub>W</sub>	74 $\pm$ 4 <sup>A</sup> <sub>W</sub>	78 $\pm$ 5 <sup>BC</sup> <sub>WX</sub>	*99 $\pm$ 8 <sup>A</sup> <sub>W</sub>	92 $\pm$ 8 <sup>AB</sup> <sub>W</sub>	81 $\pm$ 4 <sup>BC</sup> <sub>W</sub>	73 $\pm$ 4 <sup>C</sup> <sub>WX</sub>	73 $\pm$ 5 <sup>C</sup> <sub>W</sub>	69 $\pm$ 4 <sup>C</sup> <sub>W</sub>	0.0006
	$P > F$		0.0012	0.0010	0.0087	0.0001	0.0016	0.0014	0.0178	0.0003	0.0031	0.0004	0.0001	

Different superscript letters within a row or column indicate significant differences at  $P < 0.05$ . Superscripts (A–F) indicate differences within a row (amino acid effect) and subscripts (W–Z) indicate differences within a column (ingredient effect).

<sup>1</sup> Probability associated with the  $F$ -statistic.

\* Individual amino acid availability is significantly different compared with the mean amino acid availability (AA mean) within a feedstuff at the  $P < 0.05$  significance level.

The nutritional value of poultry by-product meal to fish has been variable. Protein digestibility coefficients have ranged from about 40% to more than 90% depending on the product source and species of fish (Dong *et al.* 1993; Gaylord & Gatlin 1996; Sugiura *et al.* 1998; Bureau *et al.* 1999). Results from the present trial with hybrid striped bass illustrate the potential for low digestibility of protein (55%) from poultry by-product meal. Overall amino acid availability was comparable with protein digestibility and individual amino acid availability was consistent across the amino acids tested in poultry by-product meal. Similar findings have been reported for gilthead seabream in which product quality and, specifically, protein digestibility was low from poultry meals that contained feathers (Nengas *et al.* 1999).

Performance of hybrid striped bass also has been variable when poultry by-product meal has been utilized as a major source of dietary protein. Webster *et al.* (1999) observed equivalent feed conversion and growth rates of hybrid striped bass while Webster *et al.* (2000) observed an increased feed conversion ratio from 2.0 to 2.7 when fish meal in the diets was replaced with poultry by-product meal. The authors speculated that the discrepancies between the two studies may have been attributable to differences in the proportion of processing waste included in the by-product or processing methodology that may have reduced nutrient availability.

Protein in fish solubles was 70% digestible in the present trial. However, several amino acids may be of concern when utilizing this feedstuff in diet formulations for hybrid striped bass. Most notably, the availabilities of lysine and the aromatic amino acids, phenylalanine and tyrosine, were depressed compared with other amino acids. Decreased lysine availability has been observed with other feedstuffs as lysine is highly sensitive to processing damage (Plakas *et al.* 1985; Nengas *et al.* 1999; Ljokjel *et al.* 2000).

The blended products tested in the current study (Pro-Pak 60, Pro-Pak 65, ProCon and 60FMC) have been generally marketed as potential replacements for fishmeal in animal production diets. Although protein digestibilities ranged from 47 to 63%, Pro-Pak 60 exhibited the most favourable amino acid availabilities among all amino acids tested as well as higher overall amino acid availability than protein digestibility. This was observed although overall protein digestibilities were not statistically different among the blended products. Protein digestibility in Pro-Pak has previously been reported at 56% for hybrid striped bass (Rawles & Gatlin 2000) and is comparable with the 63% observed in the current trial.

Of the plant products tested, peanut meal had the highest crude protein digestibility (80%). Moreover, amino acid

availabilities from peanut meal were generally high with one exception. Histidine availability appeared to be somewhat lower than the other essential amino acids. Similarly, histidine availability was determined to be 65% in striped bass (Small *et al.* 1999) compared with 68% in the present study with sunshine bass. All other amino acid availabilities ranged from 80 to 94% for peanut meal. Wilson *et al.* (1981) also observed a slight depression in histidine availability from peanut meal for channel catfish at 83% compared with an average amino acid availability of 88%. Generally, peanut meal appears to be a favourable feedstuff for hybrid striped bass diets if formulations compensate for the low lysine and methionine content and lower histidine availability from this product.

Histidine availability also may be problematic in other plant products processed for animal feeds. Matsumoto *et al.* (1996) found that histidine availability to yellowtail (*Seriola quinqueradiata*) was 53% compared with an average of 78% for the other amino acids measured in full-fat soybean meal. However, Wilson *et al.* (1981) observed no depression in histidine availability from soybean meal for channel catfish. De Silva *et al.* (2000) noted that there are potential species differences in amino acid availabilities from the same feedstuff. Moreover, differences in product processing, handling and storage conditions can differentially affect amino acid availability.

The digestibility of protein (43%) as well as the availability of specific amino acids (37–62%) in canola meal were relatively poor for hybrid striped bass in the current trial. Histidine and valine availability were notably reduced to 42 and 37%, respectively, compared with many of the other amino acids tested. Much higher coefficients of protein digestibility and amino acid availability have been reported for canola meal in silver perch diets (Allan *et al.* 2000).

Among the plant products tested, lysine and methionine availabilities from brewer's yeast, peanut meal and sunflower meal were notably high in hybrid striped bass. This may be due in part to the relatively low concentrations of these two amino acids in these feedstuffs. Some animal products tested in the current experiment also were low in specific amino acids, but their amino acid availabilities were not concomitantly high. This could be due to inherent differences in animal and plant derived ingredients. Most animal products undergo heating and drying processes, often severe, that plant ingredients do not (Abdel-Warith *et al.* 2001; Millamena & Golez 2001). This may be one factor contributing to differences among amino acid availabilities that cautions against broad generalizations based on relative concentrations of amino acid in different ingredients. Com-

petition for uptake among amino acids does occur while severe imbalances in dietary ratios can impair the uptake of certain amino acids, notably the branched chain amino acids as well as arginine and lysine (Wilson 2002).

It is difficult from the design of the present experiment to determine potential effects of processing on the nutritional quality of the tested feedstuffs. Differences in the proportion of various body parts included in an animal by-product along with differences in processing conditions, such as temperature and duration of heating, can potentially alter the nutritional value of a feedstuff. Such factors can greatly influence the digestibility of protein and differentially influence the availability of amino acids from a feed ingredient. Results from the current *in vivo* digestibility trial point out that caution is necessary when substituting ingredients during feed formulation, and emphasis should be placed on formulating feeds based on available amino acid needs of the animal. The values determined from the current experiments will give the feed formulator more options in formulating fish feeds based on available amino acids.

## Acknowledgements

The authors are grateful to Larry Turner, H.J. Baker Brothers, Ft. Smith, AR and Matt McCrory, Mid-South Milling, Memphis, TN for providing animal product blends; Jess Walls, Tyson Foods, Rogers, AR for providing poultry by-product meal; Jeff Johnson, Omega Protein, Hammond, LA for providing Select menhaden fish meal; Brian Langdon, Omega Protein, Reedville, VA for providing menhaden fish oil; Mike Slaymaker, Archer Daniels Midland, Decatur, IL for providing canola and sunflower meals; Bill Ventress, Sessions Inc., Enterprise, AL for providing peanut meal; Wayne Gamble, Riceland Foods, Stuttgart, AR for providing soybean meal; Gaylon Roberts, Producer's Rice Mill, Stuttgart, AR for providing rice bran; and Mike Freeze, Keo Fish Farms, Keo, AR for providing fish used in this study.

## References

Abdel-Warith, A.A., Russell, P.M. & Davies, S.J. (2001) Inclusion of a commercial poultry by-product meal as a protein replacement of fish meal in practical diets for African catfish *Clarias gariepinus* (Burchell 1822). *Aquacult. Res.*, **32**, 296–305.

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J. & Warner-Smith, R. (2000) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. *Aquaculture*, **186**, 293–310.

AOAC (1995) *Official Methods of Analysis*. Association of Official Analytical Chemists, Inc., Arlington, VA, USA.

Brown, P.B. (1995) Using whole-body amino acid patterns and quantitative requirements to rapidly develop diets for new species such as striped bass (*Morone saxatilis*). *J. Appl. Ichthyol.*, **11**, 342–346.

Bureau, D.P., Harris, A.M. & Cho, C.Y. (1999) Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **180**, 345–358.

De Silva, S.S., Gunasekera, R.M. & Gooley, G. (2000) Digestibility and amino acid availability of three protein-rich ingredient-incorporated diets by Murray cod *Maccullochella peelii peelii* (Mitchell) and Australian shortfin eel *Anguilla australis* (Richardson). *Aquacult. Res.*, **31**, 195–205.

Dong, F.M., Hardy, R.W., Haard, N.F., Barrows, F.T., Rasco, B.A., Fairgrieve, W.T. & Forster, I.P. (1993) Chemical composition and protein digestibility of poultry by-product meals for salmonid diets. *Aquaculture*, **116**, 149–158.

Fleming, J., Taylor, T., Miller, C. & Woodward, C. (1992) *Analysis of Complex Mixtures of Amino Acids using the HP 1050 Modular HPLC*. Application Note 228-212, publication no. 5091-5615E. Agilent Technologies Inc., Palo Alto, CA, USA.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509.

Fry, J.C. (1993) Bivariate regression. In: *Biological Data Analysis: a Practical Approach* (Fry, J.C. ed.), pp. 81–125. Oxford University Press, Oxford, UK.

Gatlin, D.M. III (1997) Nutrition and feeding of striped bass and hybrid striped bass. In: *Striped Bass and Other Morone Culture* (Harrell, R.M. ed.), pp. 235–251. Elsevier Science, New York, NY, USA.

Gaylord, T.G. & Gatlin, D.M. III (1996) Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). *Aquaculture*, **139**, 303–314.

Griffin, M.E., Brown, P.B. & Grant, A.L. (1992) The dietary lysine requirement of juvenile hybrid striped bass. *J. Nutr.*, **122**, 1332–1337.

Griffin, M.E., Wilson, K.A. & Brown, P.B. (1994) Dietary arginine requirement of juvenile hybrid striped bass. *J. Nutr.*, **124**, 888–893.

Keembiyehetty, C.N. & Gatlin, D.M. III (1992) Dietary lysine requirement of juvenile hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). *Aquaculture*, **104**, 271–277.

Keembiyehetty, C.N. & Gatlin, D.M. III (1993) Total sulfur amino acid requirement of juvenile hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). *Aquaculture*, **110**, 331–339.

Keembiyehetty, C.N. & Gatlin, D.M. III (1997) Dietary threonine requirement of juvenile hybrid striped bass (*Morone chrysops* × *M. saxatilis*). *Aquacult. Nutr.*, **3**, 217–221.

Kleiber, M. (1961) *The Fire of Life: An Introduction to Animal Energetics*. John Wiley and Sons, Inc., New York, NY, USA.

Kramer, C.Y. (1956) Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics*, **12**, 307–310.

Lee, S. (2002) Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegelii*). *Aquaculture*, **207**, 79–95.

Ljokjel, K., Harstad, O.M. & Skrede, A. (2000) Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows. *Anim. Feed Sci. Technol.*, **84**, 83–95.

Matsumoto, T., Ruchimat, T., Ito, Y., Hosokawa, H. & Shimeno, S. (1996) Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). *Aquaculture*, **146**, 109–119.

- Millamena, O.M. & Golez, N.V. (2001) Evaluation of processed meat solubles as replacement for fish meal in diet for juvenile grouper *Epinephelus coioides* (Hamilton). *Aquacult. Res.*, **32**, 281–287.
- Nengas, I., Alexis, M.N. & Davies, S.J. (1999) High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream *Sparus aurata* L. *Aquaculture*, **179**, 13–23.
- NRC (National Research Council) (1993) *Nutrient Requirements of Fish*. National Academy Press, Washington, DC, USA.
- Ott, L. (1977) *An Introduction to Statistical Methods and Data Analysis*. Duxbury Press, North Scituate, MA, USA.
- Plakas, S.M., Lee, T.C., Wolke, R.E. & Meade, T.L. (1985) Effect of Maillard browning reaction on protein utilization and plasma amino acid response by rainbow trout (*Salmo gairdneri*). *J. Nutr.*, **115**, 1589–1599.
- Rawles, S.D. & Gatlin, D.M. III (2000) Nutrient digestibility of common feedstuffs in extruded diets for sunshine bass *Morone chrysops* × *Morone saxatilis*. *J. World Aquacult. Soc.*, **31**, 570–579.
- Robinson, E.H., Poe, W.E. & Wilson, R.P. (1984) Effects of feeding diets containing an imbalance of branched-chain amino acids on fingerling catfish. *Aquaculture*, **37**, 51–62.
- Small, B.C., Austic, R.E. & Soares, J.H. Jr (1999) Amino acid availability of four practical feed ingredients fed to striped bass *Morone saxatilis*. *J. World Aquacult. Soc.*, **30**, 58–64.
- Sugiura, S.H., Dong, F.M., Rathbone, C.K. & Hardy, R.W. (1998) Apparent protein and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture*, **159**, 177–202.
- Sullivan, J.A. & Reigh, R.C. (1995) Apparent digestibility of selected feedstuffs in diets for hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). *Aquaculture*, **138**, 313–322.
- Tukey, J.W. (1953) *The Problem of Multiple Comparisons*. Unpublished Manuscript. Princeton University, Princeton, NJ, USA.
- Webster, C.D., Tiu, L.G., Morgan, A.M. & Gannam, A.L. (1999) Effect of partial and total replacement of fish meal on growth and body composition of sunshine bass *Morone chrysops* × *Morone saxatilis* fed practical diets. *J. World Aquacult. Soc.*, **30**, 443–453.
- Webster, C.D., Thomson, K.R., Morgan, A.M., Grisby, E.J. & Gannam, A.L. (2000) Use of hempseed meal, poultry by-product meal and canola meal in practical diets without fish meal for sunshine bass (*Morone chrysops* × *Morone saxatilis*). *Aquaculture*, **188**, 299–309.
- Wilson, R.P. (2002) Amino acids and proteins. In: *Fish Nutrition*, 3rd edn (Halver, J.E. & Hardy, R.W. eds), pp. 143–179. Academic Press Inc., San Diego, CA, USA.
- Wilson, R.P., Robinson, E.H. & Poe, W.E. (1981) Apparent and true availability of amino acids from common feed ingredients for channel catfish. *J. Nutr.*, **111**, 923–929.
- Yamamoto, T., Shima, T. & Furuita, H. (2004) Antagonistic effects of branched-chain amino acids induced by excess protein-bound leucine in diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **232**, 539–550.